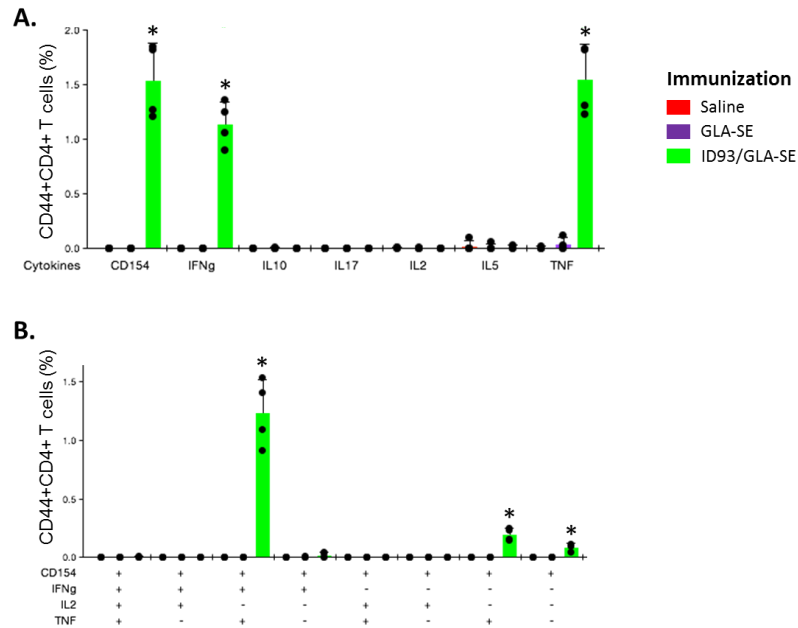
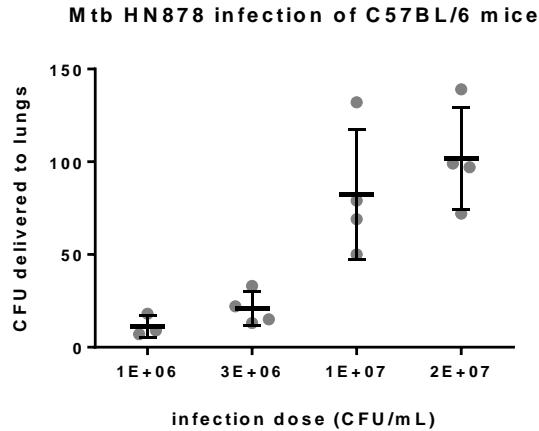


Supplemental Material



Supplemental Figure 1. ID93/GLA-SE elicits T_H1 cytokines from CD4+ T cells. C57BL/6 mice were immunized three times, at 3 week intervals. Four weeks after the final immunization, spleens were harvested from individual mice. Splenocytes were stimulated with ID93, or medium alone control, for 8 hours with Brefeldin A (GolgiStop), then stained with CD4, CD8, CD44, CD154, IFN- γ , IL-2, IL-5, IL-10, IL-17 and TNF- α fluorochrome-conjugated antibodies. Medium-only background was subtracted from ID93-stimulated samples. **(A)** Percentage of single-cytokine producing CD44+CD4+ T cells. **(B)** Percentage of poly-functional T_H1 -cytokine producing CD44+CD4+ T cells. Bars indicate the mean + SD, with dots for individual values. * $P < 0.05$ versus saline-immunized, Wilcoxon signed-rank test.



Supplemental Figure 2. Infectious dose titration for *M. tuberculosis* HN878. Four C57BL/6 mice per dose were infected with various concentrations of *M. tuberculosis* HN878 using an aerosol exposure chamber (University of Wisconsin, Madison, WI), to determine the amount of viable bacteria delivered to the lung at each dose. Twenty-four hours post-infection, the entire lung was harvested, homogenized and plated on 7H10 agar plates. Plates were incubated for two to three weeks at 37°C, 5% CO₂. Colonies were counted and the aerosol dose yielding approximately 100 CFU per mouse was selected for use. The bar shows the mean \pm SD for each concentration and dots indicate individual values.

Supplemental Table 1. Protection against *M. tuberculosis* HN878 with ID93/GLA-SE.

Lung[^] Group[#]		Log₁₀	SD	SE	Log Protection vs. Saline*	P Value
1	Saline	6.45	0.23	0.09		
2	BCG	5.49	0.22	0.08	0.96	P<0.0001
3	GLA-SE	6.17	0.24	0.09	0.28	P>0.05
4	ID93/GLA-SE	5.91	0.21	0.08	0.54	P<0.001
Spleen[^] Group[#]		Log₁₀	SD	SE	Log Protection vs. Saline*	P Value
1	Saline	5.08	0.34	0.13		
2	BCG	4.29	0.23	0.09	0.79	P<0.0001
3	GLA-SE	4.96	0.28	0.11	0.12	P>0.05
4	ID93/GLA-SE	4.55	0.18	0.07	0.52	P<0.001

*Significance was determined by one-way ANOVA with Tukey's multiple comparison test.

[^]Lungs and spleens of immunized mice were harvested 4 weeks after aerosol challenge.

[#]N = 7 mice per group.